PAGANO-LEVIN MEDIUM FOR THE ISOLATION AND IDENTIFICATION OF *CANDIDA ALBICANS**

STANLEY A. ROSENTHAL, PH.D. AND DOMENICA FURNARI, B.A.

The problem of distinguishing *Candida albicans* from other *Candida* species, and from other yeast-like organisms, is usually reserved for diagnostic mycologic laboratories, as most practicing physicians do not have the facilities to undertake such species identification.

Attempts to devise culture media upon which the growth of *C. albicans* is distinctive (1) have not always been successful (2). However, in 1958, Pagano, Levin and Trejo (3) described a medium upon which laboratory strains of *C. albicans* were distinguishable from other stock candidas and yeast strains. The indicator used in this medium was 2,3,5-triphenyltetrazolium chloride which could be reduced to insoluble, red formazans by biological activity. *C. albicans* had little ability to reduce this compound and the colonies were only light pink or white in color. Other candidas, with the exception of *C. krusei*, produced colonies much more intensely colored. *C. krusei*, however, formed colonies which were flat, dry and wrinkled as compared with *C. albicans* colonies which were raised and smooth. On this basis, cultures of *C. albicans* could be readily distinguished. These findings have been confirmed by Taschdjian in laboratory strains of yeast-like fungi (4).

The present study was designed to test, not just with laboratory cultures, but under actual clinical conditions, the value of the Pagano, Levin and Trejo medium as an aid in the isolation and identification of *C. albicans*.

METHOD

Scrapings from 95 persons with clinical candidiasis were used in this study. To ascertain whether the medium of Pagano, Levin and Trejo (PL)† is a satisfactory medium for the isolation of *C. albicans*, the scrapings obtained from each subject were inoculated onto both PL and Sabouraud-cycloheximide-chloramphenicol agar (SCC) (5). The PL tubes were examined daily for seven days. Solely on the basis of the gross appearance of the isolates, and without reference to the growth on SCC or the results of differential tests, the cultures on PL were placed into 1 of 2 categories—"*C. albicans*" or "Not *C. albicans*."

Subsequently, the primary yeast-like isolates from both PL and SCC media were transferred to rice infusion agar plates (6). Those strains showing the presence of typical chlamydospores were classified as *C. albicans*. Those strains forming filaments, but no chlamydospores, were further examined by fermentation and assimilation studies as described by Benham (7), and, in some cases, by slide agglutination (8).

RESULTS

Growth of the isolates of *C. albicans* obtained on PL was uniform. The young colonies, which first appeared 1 to 5 days after inoculation, were white, slightly raised and moist. Following 1 to 2 days of additional incubation, most of the colonies took on a very faint pink color in their central portion. After further growth the entire colony appeared to have a darker pink hue. Using these morphologic features as our criteria, these organisms were identified as *C. albicans*.

C. albicans was easily distinguishable from other yeast-like organisms and bacteria. The bacterial colonies were small and flat, while the yeast grew from the start as pink or red colonies.

PL appears to be as satisfactory an isolation medium as SCC, if not even somewhat better. Table 1 shows that in 9 instances *C. albicans* was isolated on PL and not on SCC. Isolation on SCC but not PL occurred only 5 times. However, it should be pointed out that this superiority as an isolation medium did not apply to *T. rubrum*. From the 95 cases of clinical candidiasis, isolation of *T. rubrum* was accomplished on SCC in 6 instances and only once on PL. In 2 subjects, a mixed fungous flora was revealed, with *T. rubrum* growing only on SCC and *C. albicans* only on PL.

The data appearing in Table 2 clearly indicate the value of PL in the identification of *C. albicans* colonies. From 60 of the PL tubes showing

* From the Department of Dermatology and Syphilology (Dr. Marion B. Sulzberger, Chairman) of the New York University Post-Graduate Medical School and the Skin and Cancer Unit of University Hospital, New York, N. Y.

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† The medium of Pagano, Levin and Trejo was generously supplied by Dr. John T. Groel, The Squibb Institute for Medical Research, New Brunswick, New Jersey. It is available under the name "Pagano-Levin medium."

TABLE 1

Isolation of C. albicans on Pagano-Levin medium (PL) and Sabouraud - cycloheximide - chloramphenicol agar (SCC) from 95 subjects with clinical candidiasis

C. albicans isolated on both media..	40 subjects
C. albicans isolated on PL only.....	9 subjects
C. albicans isolated on SCC only..	5 subjects
C. albicans not isolated on either medium.....	41 subjects

TABLE 2

Value of Pagano-Levin medium (PL) in the diagnosis of C. albicans

Growth on PL Diagnosed as:	No. of Strains	No. of Cultures Identified as:	
		C. albicans	NOT *C. albicans*
C. albicans	49	49*	0
Not *C. albicans*	11	0	11—*T. rubrum* (1) Bacteria (6) Saprophytic yeast (3) *C. tropicalis* (1)

* One of these isolates failed to produce chlamydospores on rice infusion agar. However, fermentative and carbon assimilation tests were characteristic for *C. albicans*.

growth, 49 were called *C. albicans* on the basis of their gross colony appearance, and all 49 proved to be *C. albicans*. Eleven of the tubes gave rise to colonies, which, on the basis of their gross characteristics, were considered not to be *C. albicans*; none of these were found to be *C. albicans*, but one proved to be another *Candida* species, *C. tropicalis*. This strain appeared on PL for the first time after 2 days' incubation as a shiny colony, faintly pink throughout. The color became slightly more intense on continued incubation.

COMMENT

The necessity of reading PL tubes at 1 or 2 day intervals to successfully identify *C. albicans* should be stressed. The color changes of the colonies from completely white to white with pink central area and finally, in some instances, to a diffusely pink colony occur rather rapidly. If readings are delayed until after growth has been present for some time, the differences in color between *C. albicans* and other yeasts may be so slight as to make a correct diagnosis difficult.

PL, under routine clinical conditions, has proven to be a useful medium for the isolation and identification of *C. albicans*. Perhaps its greatest value will be found in office mycology where the pressure of time and lack of facilities often make the differentiation between the various species of *Candida* a difficult or impossible task.

SUMMARY

Under actual clinical working conditions Pagano-Levin medium has been found to be useful for the rapid isolation and identification of *Candida albicans*.

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